Assessment of Ca^{2+} -Antagonistic Effects of SM-6586 and Its Isomers, Novel 1,4-Dihydropyridine Derivatives, by Radioligand Binding Assay

Junji Kinami, Ying-Li Qu, Hiroshi Tsuchihashi, Takafumi Nagatomo, Takashi Maniwa¹ and Akira Miyagishi¹

Department of Pharmacology, Niigata College of Pharmacy, Niigata 950-21, Japan

¹*Research Laboratories, Sumitomo Pharmaceuticals Co. Ltd., Osaka 554, Japan*

Received August 26, 1991 Accepted November 12, 1991

ABSTRACT—The Ca^{2+} -antagonistic effects of the 1,4-dihydropyridine derivative ($\pm$)SM-6586 and its optical isomers were compared with those of its two derivatives (($\pm$)SM-7297 and ($\pm$)SM-7548) and other Ca^{2+} -antagonists using a radioligand binding assay. The Ca^{2+} -antagonistic effects of the optical isomers of SM-6586 were in the order of (+) > ($\pm$) > (–)SM-6586 in both rat brain and heart. The pK_i value of (+)SM-6586 was comparable to those of nimodipine, nicardipine, nifedipine and nitrendipine. The pA_2 value for (+)SM-6586 was the highest among the SM-6586 isomers, thus suggesting that (+)SM-6586 has a potent Ca^{2+} -antagonistic effect.

SM-6586 (methyl 1,4-dihydro-2,6-dimethyl-3-[[3-*N*-benzyl-*N*-methylaminomethyl)-1,2,4-oxadiazolyl-5-yl]-4-(3-nitrophenyl)pyridine-5-carboxylate) is a novel 1,4-dihydropyridine derivative with the following actions: 1) it competitively inhibits Ca-induced contraction of rat aorta in a high K solution, 2) produces a dose-dependent reduction of blood pressure in conscious SHR, and 3) is effective in preventing the development of cerebral stroke in SHRSP (1, 2).

Recently, radiolabelled 1,4-dihydropyridine including ^3H -nitrendipine and ^3H -PN200-110 have been used to identify and characterize dihydropyridine binding sites and to assess Ca^{2+} antagonistic potencies of chemicals associated with calcium channels in the heart, brain and other tissues (3, 4). Thus, this study was designed 1) to assess the Ca^{2+} -antagonistic affinities of SM-6586 and its optical isomers to rat brain and heart and 2) to compare them with those of its two derivatives (SM-7297,

methyl 1,4-dihydro-2,4-dimethyl-3-(3-morpholinomethyl-1,2,4-oxadiazol-5-yl)-4-(3-nitrophenyl)-5-pyridinecarboxylate hydrochloride and SM-7548, methyl 1,4-dihydro-2,4-dimethyl-4-(2-nitrophenyl)-3-(3-piperidinomethyl-1,2,4-oxadiazol-5-yl)-5-pyridinecarboxylate hydrochloride) and other Ca-antagonists to determine the specific structural features of this agent necessary for its Ca-antagonistic activity using the radioligand binding assay method, and pK_i values were compared with the pA_2 values obtained from pharmacological experiments.

^3H -Nitrendipine (84 Ci/mmol) and ^3H -PN200-110 (70 Ci/mmol) were purchased from Amersham Japan Co., Ltd. and New England Nuclear Corp, Ltd., respectively. ($\pm$)SM-6586, its optical isomers and its two derivatives (($\pm$)SM-7297 and ($\pm$)SM-7548) (Fig. 1) were used in the present study.

The crude membrane fractions from the rat brain and heart were prepared as described previously (5). Monophasic displacement

Fig. 1. Chemical structures of SM-6586, SM-7297 and SM-7548. * Asymmetric carbon atom.

analysis for 1,4-dihydropyridine binding sites using the radioligand binding assay method was carried out in duplicate with ^3H -nitrendipine and ^3H -PN200-110. The membrane suspension (0.35 or 0.4 mg of protein) obtained from rat brain or heart was incubated for 45 min at 23°C with 1.5 nM of radioligand (^3H -nitrendipine or ^3H -PN200-110) in a total volume of 0.5 ml containing 60 mM Tris-HCl and 20 mM MgCl_2 (pH 7.2). At the end of the incubation period, the incubation medium was immediately filtered through a GF/C glass fiber filter. With the ^3H -nitrendipine and ^3H -PN200-110 binding, the filter in scintillation fluid (Scintizol EX-H, Dojin) was counted in a scintillation counter (Aloka LSC-700). The difference between the total and the non-specific binding, which was determined in the presence of $10\text{ }\mu\text{M}$ nifedipine, was taken as the specific binding. Protein concentrations were determined by the method of Lowry et al. (6) using bovine serum albumin as the standard. All kinetic analyses were carried out on an NEC PC-9801 computer system that performed iterative linear regression, and the K_i values of the drugs were calculated using the previously described equation (5).

The thoracic aorta of male Wistar rats, each weighing 300–350 g, was cut spirally and placed in Krebs-Henseleit solution, saturated with a 95% O_2 –5% CO_2 gas mixture and kept at 37°C . An initial resting tension of 1 g was applied to the organ, and its contractions were recorded by means of an isometric force-displacement transducer for the pharmacological experiment. The composition of Krebs-Henseleit solution was as follows: 118 mM

NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 25.0 mM NaHCO_3 , 1.2 mM MgSO_4 , 1.2 mM KH_2PO_4 and 11.5 mM glucose (pH 7.3). After a 60–90 min equilibration period, the aortic strips were incubated successively for 30 min in a Ca^{2+} -free Krebs-Henseleit solution containing 0.1 mM EGTA and then for 10 min in Ca^{2+} -free, K^+ (80 mM)-depolarizing Krebs-Henseleit solution, in which the NaCl concentration was reduced to 42.7 mM. CaCl_2 (0.03–4 mM) was added cumulatively to the organ bath, and cumulative concentration-response curves were obtained. Test drugs were added to the bathing solution 10 min before the second curve was recorded. The pA_2 values for the Ca antagonistic effects were determined by Schild plots (7).

As shown in Table 1, the Ca^{2+} -antagonistic effects of the optical isomers of SM-6586 were in the order of $(+) > (\pm) > (-)$ SM-6586. No significant difference between ^3H -nitrendipine and ^3H -PN200-110 bindings in brain and heart was observed. The pK_i value of $(+)\text{SM-6586}$ was comparable to those of nimodipine, nicardipine, nifedipine and nitrendipine. The pK_i values of its two derivatives were lower than those of $(\pm)\text{SM-6586}$. The pA_2 values of these chemicals were similar to the pK_i values.

SM-6586 belongs to the 1,4- group and the many reported 1,4-dihydropyridine analogues allow delineation of the well-defined structure-activity relationship (3, 8). This newly synthesized $(\pm)\text{SM-6586}$ definitely exhibited high displacement affinity of ^3H -nitrendipine and ^3H -PN200-110 bindings to the Ca^{2+} binding sites, and this drug completely inhibited the Ca -induced contraction of the rat aorta in a high

Table 1. pKi and pA₂ values of various Ca²⁺ antagonists

Drugs	pKi Value ³ H-nitrendipine		pKi Value ³ H-PN200-110		pA ₂ Value	Slope ^a
	brain	heart	brain	heart		
(+)-SM-6586	9.49 ± 0.19(4)	9.56 ± 0.45(5)	8.98 ± 0.13(4)	8.99 ± 0.15(5)	10.06 ± 0.06(7)	1.11 ± 0.10
(±)-SM-6586	8.88 ± 0.28(5)	9.13 ± 0.47(5)	8.57 ± 0.09(9)	8.96 ± 0.21(5)	9.76 ± 0.06(8)	1.15 ± 0.08
(-)-SM-6586	7.50 ± 0.17(6)	7.80 ± 0.18(5)	7.68 ± 0.12(6)	7.33 ± 0.15(5)	8.46 ± 0.08(6)	0.95 ± 0.12
SM-7297	7.59 ± 0.16(7)	7.31 ± 0.13(6)	7.58 ± 0.07(5)	7.65 ± 0.14(5)	8.49 ± 0.03(6)	1.04 ± 0.02
SM-7548	7.81 ± 0.16(7)	7.78 ± 0.10(6)	7.50 ± 0.20(5)	7.55 ± 0.18(5)	8.20 ± 0.03(6)	1.30 ± 0.10
Nicardipine	8.95 ± 0.23(7)	8.91 ± 0.12(5)	8.50 ± 0.15(7)	8.87 ± 0.24(6)	9.80 ± 0.06(7)	1.15 ± 0.10
Nifedipine	8.07 ± 0.22(5)	8.47 ± 0.27(5)	8.33 ± 0.27(7)	8.06 ± 0.23(7)	9.61 ± 0.04(7)	0.88 ± 0.12
Nimodipine	9.03 ± 0.16(6)	9.30 ± 0.29(6)	8.93 ± 0.15(8)	8.97 ± 0.27(6)	9.87 ± 0.07(6)	1.12 ± 0.10
Nitrendipine	8.78 ± 0.18(5)	8.71 ± 0.24(5)	8.51 ± 0.21(7)	8.89 ± 0.31(6)	10.19 ± 0.06(6)	0.96 ± 0.07

Values are means ± S.E. The numbers of experiments are indicated in parentheses. ^a: slope of the Schild plot.

K solution with a pA₂ value of 9.76, which suggests that this compound is a potent Ca²⁺ channel blocker. In addition, this compound (SM-6586) has enantiomers and the (-)-enantiomer and racemic compound have less affinity than (+)-SM-6586. Thus, calcium binding sites can apparently recognize the stereochemistry at C-4, and this may also contribute to the Ca²⁺ antagonistic effects.

(±)-SM-6586 had a much higher affinity to Ca²⁺ binding sites than its two derivatives ((±)-SM-7297 and (±)-SM-7548). The different position of the NO₂ substitute of the nitrophenyl group of (±)-SM-7548 can be seen and other differences in structure among these three chemicals is seen at the C-3 position of the 1,4-dihydropyridine ring; i.e., in (±)-SM-6586, (±)-SM-7297 and (±)-SM-7548, the C-3 position is substituted by a *N,N*-benzyl methylamino group, morpholinyl group and piperadinyl group, respectively. Therefore, we conclude that these differences in the substituent at C-3 and/or a different position of the NO₂ of the nitrophenyl group may play a crucial role for the Ca²⁺ antagonistic effects in tissues.

Nimodipine, other Ca²⁺-antagonists and (+)-SM-6586 had high pKi values, but (-)-SM-6586 had less potent displacement potencies. The difference in chemical structures between the drugs tested here seems to be in groups C-

3 and C-5 of the 1,4-dihydropyridine structure and different position of the NO₂ substituent of the nitrophenyl group. In particular, an isopropoxy carbonyl residue at C-5 and α -(methoxy)ethoxy carbonyl moiety at the C-3 of nimodipine and *N*-benzyl-*N*-methylamino-methyl-1,2,4-oxadiazoyl-5-yl residue at the C-3 of (+)-SM-6586 may be very important for the activity of the Ca²⁺ channel blocker because these large substituents at C-3 and C-5 may contribute to the Ca-antagonistic effects in addition to lipophilicity and long duration of action.

REFERENCES

- 1 Maniwa, T., Yoshida, E., Miyagishi, A. and Hara, Y.: Ca antagonistic action and antihypertensive effects of SM-6586, a novel 1,4-dihydropyridine derivative. *Japan. J. Pharmacol.* **52**, Supp. 1, 141P (1990)
- 2 Noguchi, T., Maniwa, T., Miyagishi, A. and Hara, Y.: Preventive effects of SM-6586, a novel Ca antagonist, on the development of cerebral stroke in stroke-prone spontaneously hypertensive rats. *Japan. J. Pharmacol.* **52**, Supp. 1, 141P (1990)
- 3 Triggle, D.J. and Janis, R.A.: Calcium channel antagonists. New perspectives from the radioligand binding assay. In *Modern Methods in Pharmacology*, Vol. 2, Edited by Back, N. and Spector, S., p. 1-35, Alan R. Liss, Inc., New York (1984)
- 4 Lee, H.R., Roeske, W.R. and Yamamura, H.I.: High affinity specific [³H](+)-PN 200-110 binding

- to dihydropyridine receptors associated with calcium channels in rat cerebral cortex and heart. *Life Sci.* **35**, 721–732 (1984)
- 5 Tsuchihashi, H., Nagatomo, T. and Imai, S.: Three binding sites of ^{125}I -iodocyanopindolol, i.e. β_1, β_2 -adrenergic and $5\text{HT}_{1\text{B}}$ -receptors in rat brain: Determined by the displacement and Scatchard analysis. *J. Pharmacobiodyn.* **12**, 509–516 (1989)
- 6 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275 (1951)
- 7 Arunlakshana, O. and Schild, H.O.: Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* **14**, 48–58 (1958)
- 8 Taylor, M.D., Badger, E.W., Steffen, R.P., Haleen, S.J., Pugsley, T.A., Shih, Y.H. et al.: 2-(2-Aryl-2-oxoethylidene)-1,2,3,4-tetrahydropyridines. Novel isomers of 1,4-dihydropyridine calcium channel blockers. *J. Med. Chem.* **31**, 1659–1664 (1988)